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ACCELERATED CLEAVAGE OF GLYCINE P-NITROPHENYL ESTER IN AN AQUEO--ETC(U)
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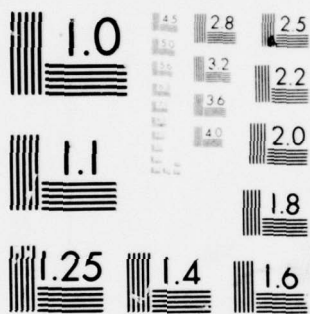
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ACCELERATED CLEAVAGE OF GLYCINE p-NITROPHENYL ESTER
IN AN AQUEOUS MEDIUM BY A CROWN ETHER COMPOUND

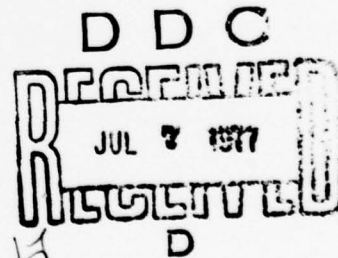
by

Ronald P. Rohrbach, Gregory D. Lyon, Licesio J. Rodriguez,
Evan L. Allred, and Edward M. Eyring

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| 20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Macrocyclic crown ether carboxylates have been investigated as catalytic host molecules for the ester cleavage reaction of guest glycine p-nitrophenyl ester ammonium ion (3-NH_3^+) in an aqueous medium. The result for 3,5-dimethyl- ylbenzoate-18-crown-5 (1c) shows p-nitrophenol release similar to that of added simple substituted benzoates, whereas 2,6-dimethylbenzoate-18-crown-5 (2c) → | | |

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→ shows a significant acceleration of the reaction rate. To aid in interpretation of these observations, rates of complexation and molar equilibrium constants (K) for closely related amino acid structures were obtained with 18-crown-6 and 18-crown-5 using an ultrasonic absorption technique. The forward and reverse rate constants were uniformly in the vicinity of $10^7 \text{ M}^{-1} \text{ s}^{-1}$ and 10^7 s^{-1} , respectively; the K values were small. A consideration of the kinetic profile for $\text{3-NH}_3^+ + \text{2c}$ is suggestive of Michaelis-Menten type catalysis dependent on initial complexation between guest and host. A strong competitive inhibition effect by Na^+ is convincing evidence of this. Structural considerations indicate that complexation of 3-NH_3^+ and 2c fixes the ester group and the carboxylate moiety in close proximity and nicely aligned for nucleophilic acyl cleavage. In contrast, the $\text{3-NH}_3^+ + \text{1c}$ system has the ester and carboxylate groups too far apart for intracomplex acyl reaction. The difference between the crown ether isomers 2c and 1c is a striking manifestation of the importance of the precise binding and catalytic site relationship necessary for catalytic activity by the host molecule.

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Accelerated Cleavage of Glycine *p*-Nitrophenyl Ester in an Aqueous Medium by a Crown Ether Compound.

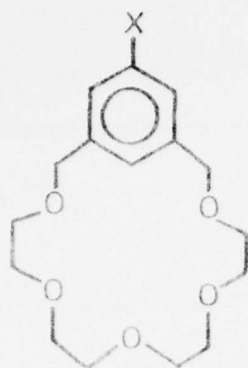
Ronald P. Rohrbach, Gregory D. Lyon, Licesio J. Rodriguez, Evan L. Allred,* and Edward M. Eyring*

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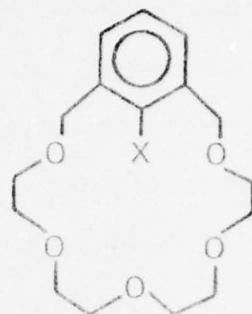
Abstract. Macrocyclic crown ether carboxylates have been investigated as catalytic host molecules for the ester cleavage reaction of guest glycine *p*-nitrophenyl ester ammonium ion (3-NH_3^+) in an aqueous medium. The result for 3,5-dimethylbenzoate-18-crown-5 (**1c**) shows *p*-nitrophenol release similar to that of added simple substituted benzoates, whereas 2,6-dimethylbenzoate-18-crown-5 (**2c**) shows a significant acceleration of the reaction rate. To aid in interpretation of these observations, rates of complexation and molar equilibrium constants (*K*) for closely related amino acid structures were obtained with 18-crown-6 and 18-crown-5 using an ultrasonic absorption technique. The forward and reverse rate constants were uniformly in the vicinity of $10^7 \text{M}^{-1} \text{s}^{-1}$ and 10^7s^{-1} , respectively; the *K* values were small. A consideration of the kinetic profile for $\text{3-NH}_3^+ + \text{2c}$ is suggestive of Michaelis-Menten type catalysis dependent on initial complexation between guest and host. A strong competitive inhibition effect by Na^+ is convincing evidence of this. Structural considerations indicate that complexation of 3-NH_3^+ and **2c** fixes the ester group and the carboxylate moiety in close proximity and nicely aligned for nucleophilic acyl cleavage. In contrast, the $\text{3-NH}_3^+ + \text{1c}$ system has the ester and carboxylate groups too far apart for intracomplex acyl reaction. The difference between the crown ether isomers **2c** and **1c** is a striking manifestation of the importance of the precise binding and catalytic site relationship necessary for catalytic activity by the host molecule.

The remarkable ability of enzymes to catalyze organic reactions has fascinated chemists for a long time. Currently there is considerable interest in the mechanistic details of such processes. Investigation, mostly with cyclodextrin model systems,¹⁻⁴ clearly points to the importance of the interrelations between complexation, breaking and making of covalent bonds, and decomplexation. This work suggests that selective complexation is crucial and that spatial orientation of the catalyzing sites is highly specific.

Because of our interests in the kinetics of complexation and in the structural factors which influence chemical reactivity, we have closely followed developments in the general area. As a consequence of this we have become interested in other possible host-guest model systems wherein the spatial position of host catalyzing sites might be varied in a known systematic way. An important requisite in this regard is a synthetically available macrocyclic system with appropriate positions which can be easily modified to incorporate catalytic sites. The crown ether type compounds immediately came to mind as attractive model systems for investigation since they had not been studied previously.⁵ Examination of molecular models (Fisher-Taylor-Hirschfelder) of a number of cyclic polyethers pinpointed an 18-crown structure which contained a 1,3-xylyl unit in the ring as being of special interest. In this system the favored conformations show the rigid aromatic group and the crown ring having substantial dihedral angles. The models also reveal that placement of catalyzing groups at the different available positions of the 1,3-xylyl moiety produces significant differences in spatial orientations relative to the complexation cavity. We chose macrocycles 1 and 2 for the preliminary testing of these considerations. Hydrolysis of amino acid ester salt was selected as the reaction for study. The p-nitrophenyl ester of glycine (3) in the form of its ammonium ion 3-NH_3^+ was picked as the guest molecule. This paper reports and discusses the results of the investigation.



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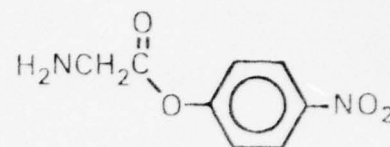


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a, X = CO₂CH₃

b, X = CO₂H

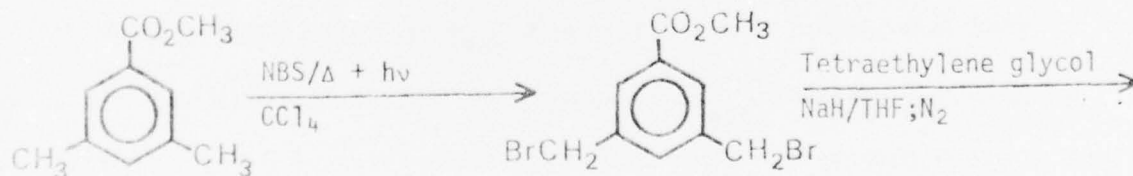
c, X = CO₂⁻



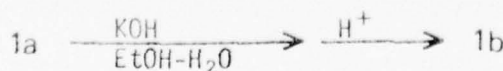
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Results and Discussion

Synthesis. The macrocyclic polyethers 1a and 1b were prepared via a procedure based on the general methods used to synthesize related crown-like compounds.^{6,7} The sequence of reaction steps using methyl 3,5-dimethylbenzoate and N-bromosuccinimide (NBS as the starting materials is presented in eq 1. The details of reaction and workup procedures are given in the experimental section. Structures assigned to 1a and 1b derive directly from the synthetic method and the expected simple proton NMR spectrum with the correct numbers and kinds of signals. The carboxylic acid derivative also showed satisfactory elemental analyses.



(1)



Polyethers 2a and 2b were synthesized from methyl 2,6-dimethylbenzoate by an analogous procedure. The NMR spectra for both compounds were in agreement with expectations. The carboxylic acid showed satisfactory elemental analyses and it had a melting point which agreed with the one reported⁵ for 2b.

Hydrolysis Kinetics. Studies of the hydrolysis of 3-NH₃⁺ were carried out in a standard reaction medium made up of 0.5% (v/v) acetonitrile/water and 0.3 M 2-picoline buffer with adjustment to a pH of 5.67 by addition of the appropriate amount of concentrated HCl. In this solution the glycine ester 3 exists largely with an -NH₃⁺ group.

Kinetic measurements of the hydrolysis of 3-NH₃⁺ were made by monitoring the development of p-nitrophenol spectrophotometrically at 360 nm. The rate of hydrolysis at 25.0°C of a solution of the standard medium made 3.5×10^{-4} M with 3 in the absence of any other added solutes was found to follow pseudo first-order kinetics. Information about the normal nucleophilic effect of carboxylate salts on the reaction was obtained by measuring hydrolysis rates of solutions containing from 0.01-0.05 M added benzoic acid (4), 2,6-dimethylbenzoic acid (5), or 3,5-dimethylbenzoic acid (6). At the pH of the standard conditions these acids exist mainly as carboxylate salts. Similarly, the effects of the modified crown compounds on the hydrolysis of 3-NH₃⁺ were determined by measuring the rate of ester cleavage in the standard medium which contained from 0.01-0.05 M of added macrocyclic acids 1b or 2b. Under these conditions 1b and 2b (pK_a in aqueous solution 3.9 and 4.8⁶, respectively) occur primarily in the form of carboxylate salts 1c and 2c, respectively. Again, pseudo first-order kinetics were found. The observed first-order rate constants (k_{obsd}) are summarized in Table I.

A comparison of the k_{obsd} measurements in Table I does not show any apparent large effect of added carboxylate ion on the hydrolysis rates of 3-NH₃⁺. However, closer inspection of the data reveals a significant divergence of effects. Additives 4, 5, 6, and crown compound 1b all tend to diminish the rates as the amount added increases. With 4-CO₂⁻, 5-CO₂⁻, and 6-CO₂⁻ the rate constants gradually decrease to ca. 0.6 of the original values at 0.05 M concentration. In contrast, crown 2c acts in the opposite direction and increases the rate constant by a factor of 2.6 over the same concentra-

Table I. Some Rate Comparisons for Hydrolysis of Glycine p-Nitrophenyl Ester Ammonium Ion (3-NH_3^+) in the Absence and Presence of Additives 1b and 2b at $25.0^\circ \text{C}^{\text{a,b}}$

| Run no. | Additives | [Additives] M | $10^4 k_{\text{obsd}}^{\text{c}}$ (s^{-1}) |
|---------|------------------|------------------|--|
| 1 | None | 0 | 3.2 |
| 2 | NaCl | 1.0 | 8.0 |
| 3 | <u>4</u> | 0.008 | 3.0 |
| 4 | <u>4</u> | 0.014 | 2.9 |
| 5 | <u>4</u> | 0.029 | 2.5 |
| 6 | <u>4</u> | 0.052 | 2.0 |
| 7 | <u>5</u> | 0.032 | 2.3 |
| 8 | <u>5</u> ; NaCl | 0.023; 1.0 | 5.3 |
| 9 | <u>6</u> | 0.050 | 1.9 |
| 10 | <u>1b</u> | 0.005 | 3.1 |
| 11 | <u>1b</u> | 0.012 | 3.1 |
| 12 | <u>1b</u> | 0.026 | 2.9 |
| 13 | <u>1b</u> | 0.057 | 2.9 |
| 14 | <u>2b</u> | 0.010 | 4.6 |
| 15 | <u>2b</u> | 0.018 | 5.6 |
| 16 | <u>2b</u> | 0.023 | 6.5 |
| 17 | <u>2b</u> | 0.038 | 7.5 |
| 18 | <u>2b</u> | 0.046 | 8.3 |
| 19 | <u>2b</u> ; NaCl | 0.019; 1.0 | 9.4 |

^aThe standard hydrolysis medium consisted of 0.5% (v/v) acetonitrile/water and 0.3 M 2-picoline buffer with the pH adjusted to 5.67 by addition of conc. HCl. ^bThe amount of 3 added corresponded to 3.5×10^{-4} M for all rate measurements. ^cEach measurement consisted of 15-20 points covering the range of ca. 5-90% reaction. The values are from a least squares treatment of this data; the correlation coefficients were all ca. 0.99.

tion range. From these considerations, it appeared likely that some kind of catalytic activity was involved with 2c. To provide further information about this, competitive inhibition of the reaction of 3-NH_3^+ in the presence of 2c and added NaCl was examined. For comparison purposes, the effects of NaCl on the rates of hydrolysis of 3-NH_3^+ and $\text{3-NH}_3^+ + \text{5}$ were also determined. The observed first-order rate constants are listed in Table I as runs 19, 2, and 8. The treatment of the data and dissection of the various effects is given below in the discussion of the cleavage mechanism.

Complexation Kinetics: Full interpretation of the hydrolysis results for 3-NH_3^+ requires information about the rate of complexation with macrocyclic polyethers. A literature survey revealed that little detailed cation-crown ether complexation rate data is available and that what is known deals mainly with metal cation systems.⁸⁻¹⁰ For the present study it was deemed desirable to obtain complexation kinetics for the organic substituted -NH_3^+ group.¹¹

Recent investigations in these laboratories have shown an ultrasonic absorption technique to be the method of choice for complexation kinetic measurements with crown ether systems.¹⁰ However, in this regard, several practical factors precluded direct examination of the above 3-NH_3^+ hydrolysis systems. These include (1) required high substrate concentrations (both polyethers and 3-NH_3^+), (2) experimental time spans sufficient for concurrent hydrolysis of the ester moiety, and (3) a solvent medium with unknown background ultrasonic properties. For these reasons, water whose ultrasonic properties are well established, and amino acids of appropriate structures were chosen for study. The 18-crown-6 and 15-crown-5 polyethers were selected as models of complexing crowns because they are readily available and because they provide simulation of the 18-membered ring and a macrocycle containing five oxygen atoms as in 1b and 2b.

The ultrasonic kinetic experiments were made using a laser acousto optical technique recently developed here.¹² Separate measurements at 25.1°C were made for glycine, α -alanine, β -alanine, γ -aminobutyric acid, and threonine in water containing 18-crown-6 polyether. Observations were also collected for glycine in the presence of 15-crown-5:

Total ultrasonic absorption data,¹³ expressed as α/f^2 in $\text{Np cm}^{-1} \text{s}^2$, were obtained and analyzed in terms of relaxational and nonrelaxational contributions. Only one relaxation process was detected for all cases investigated in the frequency range examined (15-205 MHz). The best fit of the data was achieved by using the one relaxation expression given in eq 2.¹⁴ Here α is the ultrasonic absorption coefficient, f

$$\alpha/f^2 = A[1 + (f/f_R)^2]^{-1} + B \quad (2)$$

is the experimental frequency, f_R is the relaxation frequency, and A and B are the relaxation amplitude and the background absorption, respectively. The three parameters to be calculated A, B, and f_R were varied in the fitting process and the best values which minimized the root mean square deviation between the experimental and calculated α/f^2 were used. The results are summarized in Table II.

As shown in Table II, solutions at the higher concentrations of amino acid and crown ether gave B values which exceeded the α/f^2 of $22 \times 10^{-17} \text{ Np cm}^{-1} \text{s}^2$ reported¹⁵ for water at 25°C. In the case of the most concentrated solution of threonine and crown ether examined B reached $42.2 \times 10^{-17} \text{ Np cm}^{-1} \text{s}^2$. Additional measurements showed these background absorptions to be consistent with those found for aqueous solutions containing the same concentrations of amino acids but no crown ether. Such high background absorptions can be attributed to several effects which include increased viscosity, changes in water structure, and/or the presence of additional unrelaxed processes.¹⁶ In regard to the last of the possible causes, detailed analysis of the data showed that a process other than complexation caused relaxation was observed only with the aqueous threonine solutions. For this case the relaxation frequency was estimated to occur at ca. 250 MHz. Comparison of the amplitudes¹⁷ with the corresponding threonine concentrations showed that a direct proportionality exists.¹⁸ This kind of correlation is suggestive of a relaxation due to a monomolecular process.¹⁹ It is reasonable to ascribe such behavior for threonine to intramolecular proton transfer²⁰ or to some conformational equilibrium.²¹ This extra relaxation process is not expected to interfere with the threonine-crown ether complexation since the two processes are separated in fre-

Table II. Relaxation Parameters from Computer Analysis for Aqueous Amino Acid-Crown Ether Complexation at 25.1°C^{a,b,c}

| Amino Acid | [Crown] ₀ M | [Amino acid] ₀ M | f_R , MHz | $10^{17}A_{Np}$ cm^{-1}s^2 | $10^{17}B_{Np}$ cm^{-1}s^2 | $10^{18}d_{\text{rms}}$ |
|--------------------------------|---------------------------|--------------------------------|-------------|---|---|-------------------------|
| 18-Crown-6 | | | | | | |
| Glycine | 0.0421 | 0.461 | 25.4 | 48.1 | 22.1 | 0.6 |
| | 0.0601 | 0.658 | 29.5 | 58.2 | 21.9 | 1.2 |
| | 0.0751 | 0.822 | 30.4 | 63.8 | 22.6 | 1.1 |
| | 0.0939 | 1.03 | 33.3 | 67.4 | 22.4 | 1.0 |
| α -Alanine | 0.105 | 0.840 | 16.6 | 124.0 | 23.1 | 1.3 |
| | 0.112 | 1.13 | 19.8 | 85.8 | 25.2 | 1.4 |
| | 0.118 | 1.42 | 21.9 | 64.4 | 25.9 | 0.9 |
| β -Alanine | 0.105 | 0.697 | 19.0 | 101.0 | 22.3 | 0.8 |
| | 0.126 | 1.06 | 23.0 | 67.1 | 24.5 | 1.3 |
| | 0.147 | 1.43 | 26.3 | 55.1 | 26.0 | 1.5 |
| γ -Aminobutyric Acid | 0.156 | 0.470 | 17.6 | 186.7 | 23.8 | 1.9 |
| | 0.146 | 0.626 | 18.9 | 146.7 | 24.2 | 1.9 |
| | 0.131 | 1.05 | 21.9 | 92.8 | 28.0 | 1.0 |
| Threonine | 0.0617 | 0.654 | 13.5 | 57.0 | 29.0 | 1.5 |
| | 0.0792 | 0.842 | 15.0 | 77.0 | 32.8 | 1.7 |
| | 0.132 | 1.40 | 18.0 | 86.6 | 42.5 | 1.5 |
| 15-Crown-5 | | | | | | |
| Glycine | 0.112 | 1.39 | 12.1 | 62.0 | 27.1 | 1.8 |
| | 0.112 | 2.10 | 18.0 | 34.7 | 29.7 | 1.2 |
| | 0.112 | 2.40 | 21.6 | 26.6 | 30.8 | 1.1 |

^aAll symbols are defined in the text. ^bThe calculated parameters f_R , A , and B were varied in the fitting process and the best values which minimized the root mean square deviation between the experimental and calculated α/f^2 were used. ^cSee reference 13. ^dRoot mean square deviation.

quency by over 200 MHz.

Prior studies with aqueous solutions of 18-crown-6¹⁰ and 15-crown-5²² showed that these polyethers exhibited relatively low amplitude concentration independent relaxations at 101 and 22.9 MHz, respectively. In each case the process was attributable to a conformational rearrangement of the macrocycle. The equilibrium involved was shown^{10,2} not to affect the interpretation of the complexation kinetic data since the complexing conformation of both crown ethers is favored in the solution by a factor of 10^2 . Therefore, the complexation process can be described mechanistically by eq 3 where C, A, and C·A refer to the crown ether, amino acid, and amino acid-crown ether complex, respectively. For this formulation the reciprocal relaxation time is given by eq 4. Here τ is the observed relaxation time, f_R is the experimental relaxation frequency, $[C]$ is the



$$\tau^{-1} = 2\pi f_R = k_f([C] + [A]) + k_r \quad (4)$$

equilibrium concentration of crown ether, and $[A]$ is the equilibrium concentration of amino acid.

Since information pertaining to the molar equilibrium constant ($K=k_f/k_r$) is necessary for determination of the rate constants k_f and k_r , spectrophotometric measurements of K values of the amino acid-crown ether systems were attempted. Due to the weak interaction between amino acid and crown ether and to the lack of a spectrally sensitive chromophore only an upper limit estimate of $K < 10 \text{ M}^{-1}$ could be obtained.

In the absence of experimental K values an iteration technique²³ was employed to determine k_f and k_r from the relaxation observations. This procedure consisted of fitting eq 4 with experimental relaxation data and the initial concentrations of amino acid and crown ether in lieu of the unknown quantities $[A]$ and $[C]$. The resulting slope and intercept allowed for calculation of a first approximation equilibrium constant. This value was used to compute new amino acid and crown ether concentrations. These were then put through a fitting treatment to give a second estimated equilibrium constant.

All of the data became self-consistent after the fifth cycle. The final k_f , k_r , and kinetically determined K values are given in Table III. It can be seen that all calculated equilibrium constants are compatible with the estimate made spectrophotometrically.

The near uniformity in the values of the calculated equilibrium constants for amino acid-crown ether complexation (Table III) is of particular interest. Other work where equilibrium constants were determined experimentally for 18-crown-6 complexation with different substituted ammonium ion series has shown that equilibrium constants of similar magnitude are found within a given structural set.¹¹ It is also interesting that all of the amino acids examined here have complexation rate constants which are of the same order of magnitude. The small variations in rate can be accounted for reasonably by differences in such factors as steric interaction, solvation, and hydrogen bonding.

Concerning the Cleavage Reaction of 3-NH_3^+ : Quantitative assessment of any special cleavage reaction of 3-NH_3^+ by the carboxylate moiety of 1c and 2c requires estimation of the intrinsic hydrolysis rate constant (k_{int}) which is to be expected from inclusion of an aryl carboxylic acid. Since carboxylate ions catalyze ester hydrolysis²⁴ it is necessary to ascertain the normal response of the 3-NH_3^+ rate to added aryl carboxylate. In this regard, benzoic acid (4) was used as a model for evaluating the nature of the effect. As already noted, small decreases in the rate constant occurred with increased addition of 4.²⁵ The data plotted as k_{obsd} vs. [4] shows a good straight line which, when fitted by a least squares treatment, may be expressed mathematically as eq 5. If it is assumed that the same linear dependence will hold for other aryl carboxylic acid additives then k_{obsd} and k_{int} are the same and eq 6 is a generalized expression where [Add] is the additive concentration. The validity of this assumption was tested by com-

$$k_{\text{obsd}} = (-2.3 \times 10^{-3} \text{ s}^{-1} \text{ M}^{-1}) [\text{4}] + 3.2 \times 10^{-4} \text{ s}^{-1} \quad (5)$$

$$k_{\text{int}} = (-2.3 \times 10^{-3} \text{ s}^{-1} \text{ M}^{-1}) [\text{Add}] + 3.2 \times 10^{-4} \text{ s}^{-1} \quad (6)$$

Table III. Self-consistent Rate and Kinetic Equilibrium Constants for Aqueous Amino Acid-Crown Ether Complexation at 25.1°C^a

| Amino acid | $k_f, 10^7 M^{-1} s^{-1}$ | $k_r, 10^7 s^{-1}$ | K, M^{-1} |
|-----------------------------|---------------------------|--------------------|---------------|
| 18-Crown-6 | | | |
| Glycine | 8.4 ± 1.0 | 12.2 ± 1.0 | 0.7 ± 0.1 |
| α -Alanine | 6.1 ± 0.7 | 5.4 ± 0.8 | 1.1 ± 0.3 |
| β -Alanine | 6.6 ± 0.5 | 7.2 ± 0.5 | 0.9 ± 0.1 |
| γ -Aminobutyric acid | 5.1 ± 0.2 | 8.3 ± 0.2 | 0.6 ± 0.1 |
| Threonine | 3.8 ± 1.3 | 6.0 ± 1.3 | 0.6 ± 0.4 |
| 15-Crown-5 | | | |
| Glycine | 5.6 ± 0.8 | <2 | >2 |

^aCalculated from the ultrasonic absorption experimental data by the iteration technique described in the text (also see reference 23).

paring calculated k_{int} and experimental k_{obsd} values for added dimethylbenzoic acids 5 and 6 in $3-NH_3^+$ hydrolysis. In both cases $k_{obsd} - k_{int}$ values were equal to zero within experimental uncertainty. These results indicate that it is reasonable to use eq 6 to estimate k_{int} for 1b and 2b since they have aryl rings with similar substitution. The relevant facts are illustrated graphically in Figure 1 by plotting $(k_{obsd} - k_{int})$ vs. [Add].

For $3-NH_3^+$ reaction with 0.05 M added 1b k_{obsd} is found to be only slightly larger than k_{int} . In contrast, $3-NH_3^+$ reaction with added 0.05 M 2b occurs with rate acceleration and k_{obsd} is significantly larger than k_{int} . It is evident from the plots of these effects shown in Figure 1 that 2b is the cause of a special kind of effect.

The perceptible curvature in the reaction profile of $3-NH_3^+ + 2b$ shown in Figure 1 is suggestive of a Michaelis-Menten type catalysis²⁶ which depends on initial complex formation between 2c and the substrate. Several other pieces of information are supportive of this. Complexation between crown ethers and monosubstituted ammonium ions is well established.^{5,6,11,27,28} The very rapid attainment of equilibrium and the small equilibrium constant values found for crown ether-ammonium ion complexes in aqueous media (vide supra) are justification for employing the steady-state approximation. It is clear from the kinetic data in Table III that the initial complexation rate between 2c and $3-NH_3^+$ will exceed the initial $3-NH_3^+$ hydrolysis rate by many powers of ten and thus allow 2c to play the role of a catalyst.

Additional information about the nature of the special effect derives from the result observed when NaCl is added to the $3-NH_3^+ + 2c$ reaction. The pertinent experiments are entries 1, 8, and 19 in Table I. In the absence of NaCl, $100(k_{obsd} - k_{int})/k_{obsd}^0$ shows a rate enhancement of 94% with 0.019 M added 2b.²⁹ When 1 M NaCl is included in a reaction mixture of the same concentrations, $100[(k_{obsd} - k_{int})/k_{obsd}^0]^{NaCl}$ shows that the rate enhancement is decreased by ca. 50%.³⁰ The lowering of the rate is attributable to competitive inhibition by Na^+ ion.³¹ This phenomenon is very strong evidence that an intermediate complex is on the reaction pathway.

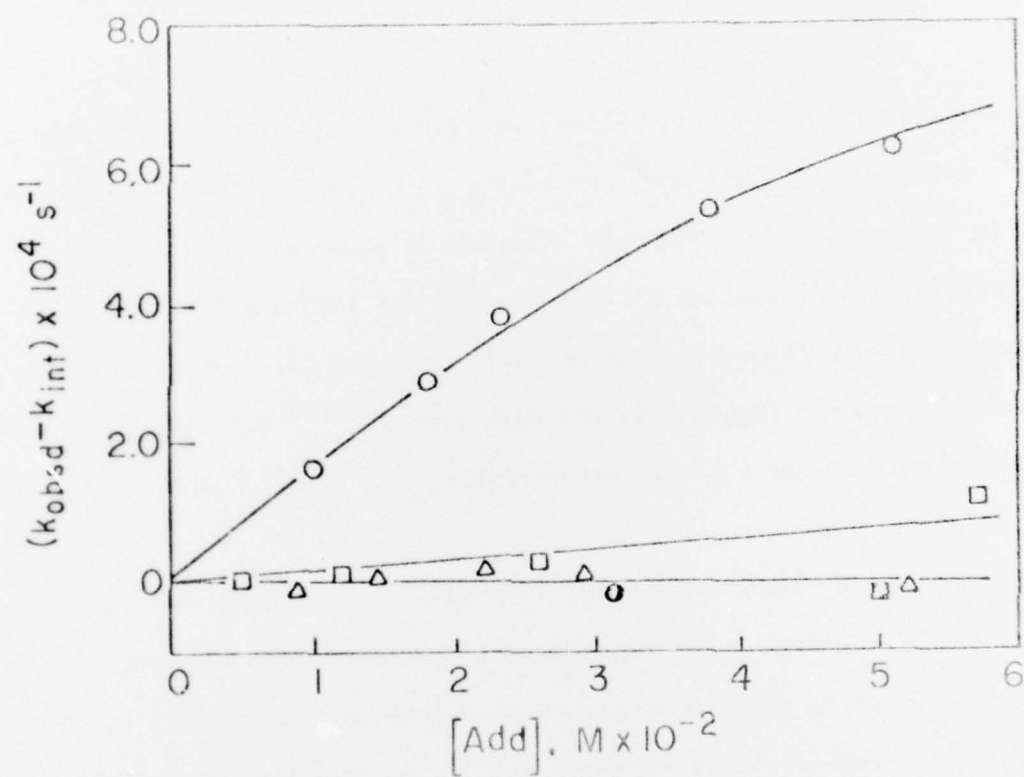
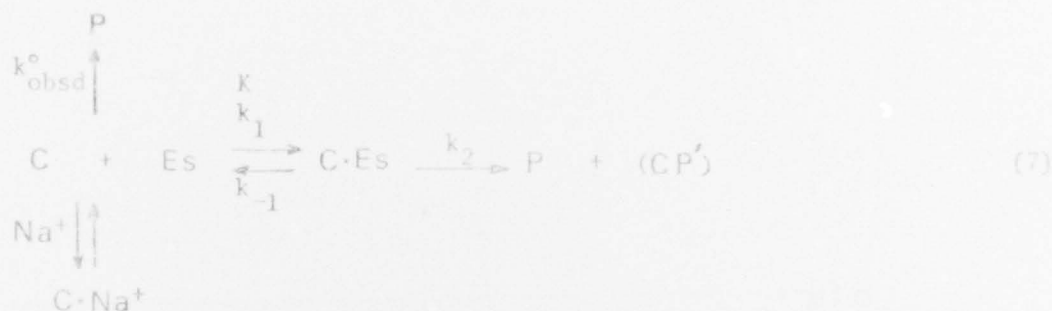


Figure 1. Plot of $(k_{\text{obsd}} - k_{\text{int}})$ against $[\text{Add}]$ at 25.0°C based on eq 6 and the data from Table I. Additives are: \triangle , benzoic acid; \bullet , 2,6-dimethylbenzoic acid; \blacksquare , 3,5-dimethylbenzoic acid; \circ , crown ether 2b; \square , crown ether 1b.

A minimal reaction scheme which accommodates these facts is shown in generalized form by eq 7. For the present case C is 2c, Es is 3-NH₃⁺, C·Es stands for the active crown ether-ammonium ion complex, C·Na⁺ is the complex from competitive inhibition,³²



P is the observed product *p*-nitrophenol, and (CP') represents the other product(s) (vide infra).³³ A Lineweaver-Burk plot³⁴ of $1/(k_{\text{obsd}} - k_{\text{int}})$ for 2b vs. $1/[\text{2b}]$ from the kinetic data in Table I yields a straight line with a slope of $(k_2 K)^{-1}$ and a y intercept of $1/k_2$ (Figure 2). Evaluation of this gives $K = 5.1 \text{ M}^{-1}$ and $k_2 = 3.3 \times 10^{-3} \text{ s}^{-1}$. This K value compares very favorably with the data obtained for similarly substituted ammonium ions and 18-crown-6 (Table III) and with reports^{32,35} for ammonium ion and 18-crown-6. It is appropriate to show a contribution from k_{obsd}° in eq 7 since k_2 is larger by only a factor of 10.

The kinetic study does not provide information concerning the intimate details of the nature of the cleavage reaction occurring in the active C·Es complex. However, scale molecular models can be used to develop a plausible partial picture of what may be involved. Manipulation of models of 3-NH₃⁺ and 2c to form the complex shows that the three hydrogens attached to nitrogen can hydrogen bond alternate oxygens in the 2c cavity and result in fixing the ester group in close proximity to the carboxylate group.³⁶ This is illustrated schematically by structure 7.³⁷ In this arrangement the carboxylate and ester groups are nicely aligned for the occurrence of nucleophilic acyl substitution. Such reaction can be expected to proceed through a tetrahedral intermediate to anhydride structure 8 and on to *p*-nitrophenol. Unfortunately, limitations of the experimental system made it impractical to seek evidence for the formation of 8. In this connection,

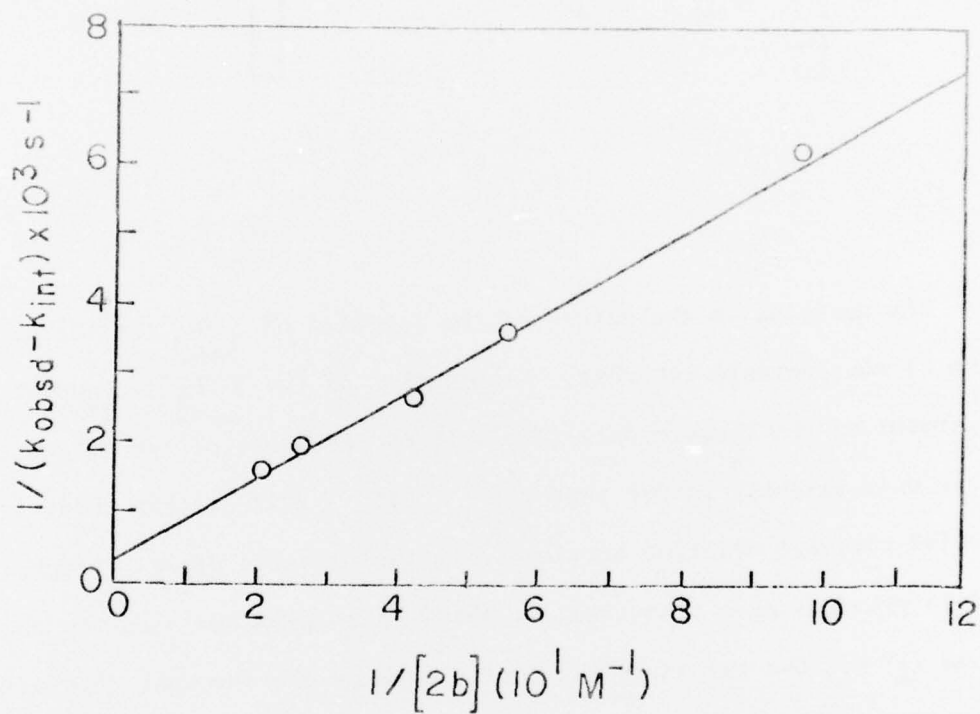
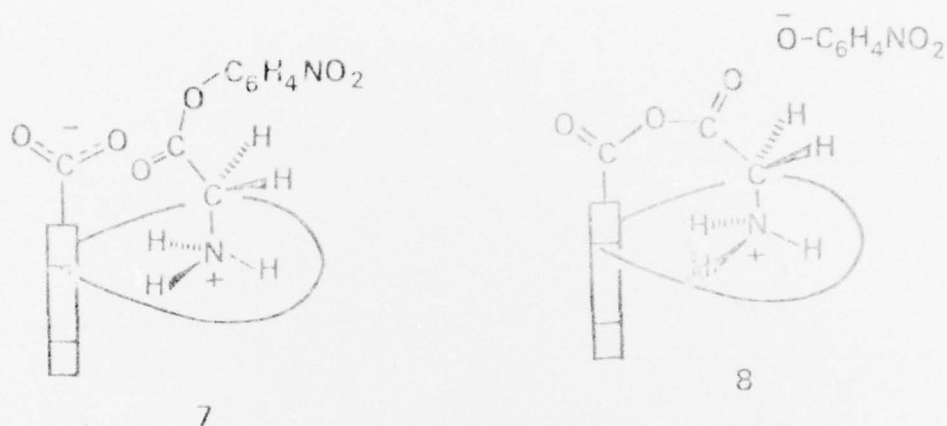


Figure 2. Lineweaver-Burk plot of $1/(k_{\text{obsd}} - k_{\text{int}})$ vs. $1/[2b]$ for the $\text{3-NH}_3^+ + 2b$ cleavage reaction.

it is likely that the anhydride intermediate with the strongly electron withdrawing $-\text{CH}_2\text{NH}_3^+$ group will hydrolyze rapidly³⁸ to 2c and glycine.³³



The question of the nature of the reaction of 3-NH_3^+ in the presence of 1c is also of considerable interest. Examination of the $\text{3-NH}_3^+ + \text{1c}$ plot in Figure 1 and treatment of the kinetic data (Table I) on the basis of the scheme in eq 7 shows that k_2 is only slightly larger than k_{obsd}^0 . It is evident that there is little, if any, special cleavage reaction ascribable to the presence of 1c. Inspection of molecular models provides an explanation for this. A complex analogous to the one described above (7) has the carboxylate and ester groups too far apart for effective intracomplex nucleophilic acyl substitution. For the $\text{3-NH}_3^+ + \text{1c}$ reaction system, the available information is indicative of ester cleavage which takes place mostly via the normal pathway for hydrolysis with added aryl carboxylate.

The markedly different behavior of the macrocyclic isomers 2c and 1c in the amino acid ester cleavage reaction is a striking manifestation of the importance of the precise binding and catalytic site relationship necessary for catalytic activity by a complexing host molecule. This finding is especially germane to chemical reactions where complexation plays a central role. A case in point is the current use of model systems to study complicated biological processes such as enzyme catalysis and inhibition. The

present work and that of Cram and Choa⁵ suggest that appropriate macrocyclic polyethers can serve useful roles as surrogates for naturally occurring host compounds in the study of biochemical reactions. Regarding this, the polyethers are totally synthetic, and therefore capable of being designed to scrutinize specific reactivity factors. In another aspect, crown ether catalysts are of obvious interest in chemical transformations such as the degradation of carboxylic acid derivatives. It is apparent that further inquiry in this new area of host-guest complexation is in order.

Experimental Section.

All ¹H NMR spectra were taken on a Varian EM-390 spectrometer and chemical shifts are δ(ppm) from internal tetramethylsilane. Melting points were taken on a Thomas-Hoover apparatus and are uncorrected.

3,6,9,12,15-Pentaoxabicyclo[15.3.1]heneicosa-1(21),17,19-triene-19-carboxylic Acid³⁹ (Crown Ether 1b). The methyl ester of 3,5-dimethylbenzoic acid was prepared by esterification with methanol and a trace of sulfuric acid. A solution of 25 g (0.13 mol) of the ester and 60 g (0.34 mol) of N-bromosuccinimide (NBS) in 700 mL of CCl₄ was stirred under reflux and irradiated with a 200-W flood lamp for 3 h. When the reaction was complete, the white precipitate floating on the surface was filtered off and the solvent was removed under vacuum. The residue was crystallized from absolute ethanol to give 13 g (33%) of 3,5-bis(bromomethyl)benzoic acid, mp 81-85°C: ¹H NMR (CDCl₃) 68.00 (s, 2, ArH), 7.61 (s, 1, ArH), 4.50 (s, 4, CH₂Br), 3.91 (s, 3, CH₃).

To a stirred slurry of 26.7 g (1.1 mol) of sodium hydride in 100 mL of refluxing tetrahydrofuran (THF) under nitrogen was added dropwise over a several hour period separate solutions of 26.7 g (0.08 mol) of the above dibromide in 1 L of THF and 19.4 g (0.09 mol) of freshly distilled tetraethylene glycol in 1 L of THF. The mixture was refluxed for 24 h under nitrogen, cooled, filtered from the salts, and the filtrate concentrated under vacuum. The concentrate was taken up in CHCl₃, washed with salt water, dried, and evaporated. The residue was chromatographed on alumina and the crude ester was eluted with benzene. This material was rechromatographed on silica gel with 50% (v) ether-CH₂Cl₂. Solvent removal gave crystalline 1a which was recrystallized from

hexane-CH₂Cl₂, mp 72-74°C: ¹H NMR (CDCl₃) δ7.95 (s,1,ArH), 7.83 (s,2,ArH), 4.65 (s,4,ArCH₂O), 3.88 (s,3,CH₃), 3.71 (s,16,CH₂CH₂). Anal. Calcd for C₁₈H₂₆O₇: C,61.02;H,7.34. Found: C,61.19;H,7.08.

Ester 1a was hydrolyzed with KOH in aqueous ethanol. This mixture was acidified with aqueous HCl and the solvent was removed under reduced pressure. The residue was chromatographed on silica gel with 50% (v) ethanol-CHCl₃ eluent. Solvent removal gave solid crown ether 1b which was recrystallized from hexane-CH₂Cl₂, mp 80-81°C: ¹H NMR (CDCl₃) δ7.99 (s,1,ArH), 7.88 (s,2,ArH), 4.69 (s,4,ArCH₂O), 3.69 (s,16,CH₂CH₂). Anal. Calcd for C₁₇H₂₄O₇: C,60.00;H,7.09. Found: C,59.91;H,7.08. The pK_a for 1b, measured by the method of half-neutralization, was found to be 3.9 ± 0.1.

3,6,9,12,15-Pentaoxabicyclo[15.3.1]heneicosa-1(21),17,19-triene-21-carboxylic Acid³⁹ (Crown Ether 2b). The methyl ester of 2,6-dimethylbenzoic acid was prepared by reaction of the acid with diazomethane in the usual way. The ester was treated with NBS as described above to give 2,6-bis(bromomethyl)benzoic acid: ¹H NMR (CDCl₃) δ7.37 (s,3,ArH), 4.59 (s,4,CH₂Br), 3.99 (s,3,CH₃).

Application of the above procedure to the 2,6-dibromide and tetraethylene glycol gave ester 2a: ¹H NMR(CDCl₃) δ7.34 (s,3,ArH), 4.60 (s,4,ArCH₂O), 3.91 (s,3,CH₃), 3.53 (m,16,CH₂CH₂). Alkaline hydrolysis of 2a followed by acidification produced crown ether 2b, mp 101-102.5°C (lit.⁶ 100-101°C): ¹H NMR(CDCl₃) δ10.68 (broad s,1,CO₂H), 7.19 (m,3,ArH), 4.68 (s,4,ArCH₂O), 3.76-3.58 (m,16,CH₂CH₂). Anal. Calcd for C₁₇H₂₄O₇: C,60.00; H,7.09. Found: C,59.92;H,7.02.

Glycine p-Nitrophenyl Ester (3-NH₃⁺). The p-nitrophenyl ester of glycine (3) was purchased from Sigma Chemical Co. as N-carbobenzoxyglycine p-nitrophenyl ester. Treatment of this derivative with HBr in acetic acid gave the bromide salt of 3-NH₃⁺.

Amino Acids. The amino acids used for the ultrasonic absorption measurements were commercial samples as indicated: glycine (Matheson, Coleman and Bell Chemical Co., 99.5% pure); DL-alanine (α-isomer) (J. T. Baker Chemical Co.); β-alanine (Aldrich Chemical Co., 98%); DL-threonine (Eastman Organic Chemicals); and 4-aminobutyric acid (Aldrich Chemical Co., 97%).

Benzoic Acids. The benzoic acids used as additives in the hydrolysis studies of 3-NH_3^+ were commercial samples as indicated: benzoic acid (4) (Baker and Adamson, reagent special standard); 2,6-dimethyl benzoic acid (5) (Aldrich Chemical Co., 97%); and 3,5-dimethylbenzoic acid (6) (Aldrich Chemical Co., 97%).

Kinetics of Hydrolysis of 3-NH_3^+ . All kinetic measurements were made with a Cary 14 recording uv-vis spectrophotometer equipped with a thermostated cell compartment maintained at $25.0 \pm 0.1^\circ\text{C}$. The cleavage reaction of 3-NH_3^+ was followed by monitoring the appearance of p-nitrophenol at 360 nm. The reaction medium was standardized and consisted of 0.5% (v/v) acetonitrile/water and 0.3 M 2-picoline buffer with the pH adjusted to 5.67 by addition of the necessary amount of concentrated HCl. This solution was prepared with deionized, redistilled water and spectroquality acetonitrile (Matheson, Coleman and Bell Chemical Co.). The reaction was initiated by adding enough 3-NH_3^+ stock solution to the standard medium so that the substrate concentration was $3.5 \times 10^{-4}\text{M}$. Other additives were included at the concentrations shown in Table I. All rate measurements showed good first-order behavior. Each measurement consisted of 15-20 absorbance readings covering the range of ca. 5-90% reaction. The infinite absorbance values were obtained after at least seven half-lives. The rate constants were determined from plots of $\log(A_\infty - A)$ vs. time.

Ultrasonic Absorption Kinetic Measurements. Ultrasonic absorption measurements for the various amino acid-crown ether systems were made at $25.1 \pm 0.1^\circ\text{C}$ over an acoustic frequency range from 15 to 205 MHz. A laser acousto optical technique developed in this laboratory was used.¹² The collection of data was facilitated by interfacing to a Digital Equipment Corp. PDP11/10 computer. The argon ion laser was operated at the 514.5 nm green line and the piezoelectric acoustic transducer element was a gold plated 5 MHz fundamental frequency, X-cut quartz crystal which was driven at odd harmonics over the frequency range.

Solutions were prepared using deionized, redistilled water. The 18-crown-6 and 15-crown-5 purchased from Parish Chemical Co. were purified as described earlier.¹⁰

Stock solutions of polyether prepared by weight were used to prepare all sample solutions by volume. The pH values of the aqueous amino acid-crown ether solutions were up to 5% higher than those at the respective isoelectric points (5.97 for glycine, 6.11 for α -alanine, 6.90 for β -alanine, 7.33 for γ -aminobutyric acid, 5.64 for threonine) at 25°C; the calculated total concentrations of zwitterion remained practically the same as the total amino acid concentration.

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Supplementary Material Available: Listing of the ultrasonic absorption α/f^2 data (Appendix I) for the various aqueous amino acid-crown ether solutions (7 pages). Ordering information is given on any current masthead page.

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